

## **FDDU Procedures for Data Analysis and Interpretation of DNA Samples using GeneMapper® ID-X Expert System Software**

### **1 Scope**

These procedures apply to DNA personnel who perform data analysis, interpretation, and technical review of the DNA typing results from FDDU samples using the Applied BioSystems (AB) GeneMapper® ID-X (GMIDX) Expert System Software as well as the import of the DNA profiles into the Sample Tracking and Control System (STACS) software. These procedures apply to the interpretation of samples amplified using either the Identifiler® Direct (IDD) or GlobalFiler® Express (GFE) Amplification Kit and analyzed on an AB 3730 DNA Analyzer, or an AB 3500xL Genetic Analyzer.

### **2 Equipment/Materials/Reagents**

STACS Software (STACS DNA Inc.), version 6.4 or higher

GeneMapper® ID-X Software (Applied BioSystems), version 1.5 or higher (For IDD) or version 1.6 or higher (For GFE)

### **3 Standards and Controls**

See the Interpretation Guidelines Section of this document for the Evaluation of Internal Size Standards, Allelic Ladders and Control Samples.

### **4 Quality Control Procedures**

#### **4.1 Random Reanalysis of FDDU DNA Samples**

FDDU samples reanalyzed during the year as part of the FDDU Procedures for CODIS Candidate Match Confirmation demonstrate random reanalysis.

#### **4.2 GeneMapper® ID-X Quarterly Performance Check**

The GMIDX Software must undergo a performance check on a quarterly basis. These checks will be in regular intervals to ensure that at least four (4) such challenges, spaced approximately three months apart, are made to the system each calendar year. A minimum of 200 FDDU samples and corresponding quality controls will be processed through the software as detailed in these standard operating procedures on each approved amplification kit/platform combination. All samples and controls, both acceptable and unacceptable, will be evaluated to ensure that the software is still performing at optimum levels. If there are any samples that were deemed

acceptable that should have been flagged for manual review, the incident will be recorded and reviewed further to find the source of the inconsistency. If it is determined that the expert system made a mistake (i.e., made an incorrect allele call), the expert system functionality will not be used to review FDDU sample data until the cause of the mistake is determined and the appropriate expert system settings adjusted as necessary. All records will be maintained.

## 5 Post PCR Review

The STACS *Post PCR Review* module provides an optional mechanism for the preliminary review of genetic analysis data generated by the AB 3730 or AB 3500xL. The preliminary review may be limited to a general evaluation of the samples/controls and the overall quality of the plate (e.g., Loss of Resolution [LOR]) or it may include a more detailed review resulting in sample(s) being sent back for rework.

**5.1** Within STACS, select the plate to be reviewed.

### 5.2 Analyzing a GeneMapper® ID-X Project

**5.2.1** Open the GMIDX Software if not already open, browse to the folder containing the sample files to be analyzed and add samples.

**5.2.2** Ensure the correct *Table Setting* is selected.

**5.2.3** The following field settings are available for use based on the instrument and kit:

Amp Kit	Instrument	Analysis Method	Panel	Size Standard
Identifiler Direct	3730	FBI GMIDX 3730 ID Direct	Identifiler_Direct_v1.2x	GS600 LIZ
		FBI GMIDX 3730 ID Direct 50 RFU		
GlobalFiler Express	3500xL	FBI GMIDX 3500 GFE	GlobalFiler_Express_v1.2x	GS600_LIZ+ Normalization_(60-460)
		FBI GMIDX 3500 GFE (75RFU)		

**5.2.4** Evaluate the electronic data in GMIDX using the *Raw Data* tab for each Combo Control (Negative) to verify the presence of a primer peak in the raw data.

**5.2.5** The analysis method settings can be viewed and, if needed, the range(s) adjusted by selecting *Analysis Method Editor* (icon with a small broad blue peak) from the tool bar on the top of the screen. The analysis settings for each kit and instrument combination are maintained with the validation and/or performance check records. Normalization is applicable to all GFE analysis.

**5.2.6** Analyze the sample files.

### **5.3 Allelic Ladder Analysis Summary**

This summary only appears if one or more of the allelic ladders have been identified as unacceptable by GMIDX.

Refer to the Interpretation Guidelines section for the review of allelic ladders.

GMIDX analysis requires the presence of at least one allelic ladder in each project. Allelic ladders with yellow and/or red quality indicators must be evaluated.

**5.3.1** Select all ladder(s) that have either a yellow triangle or a red stop sign present in the Sizing Quality (*SQ*) column.

**5.3.2** Select the “Size Match Editor” icon (icon with three red peaks) from the tool bar.

**5.3.3** Review all of the ladder files verifying the GS600 peaks (See Figure 1 or 2 in Interpretation Guidelines).

**5.3.4** Override the *SQ* of any acceptable ladder.

**5.3.5** Verify all flagged allelic ladders have been labeled with the correct allele designations (See Figure 3 or 4 in Interpretation Guidelines). If a ladder requiring manual review is deemed acceptable, the composite genotype quality of the ladder must be overridden.

**5.3.6** If an allelic ladder failed or is of poor quality (e.g., allelic ladder peaks are not labeled correctly or an extraneous peak is present within the allelic ladder), it may be removed from the project. Alternatively, the option to continue the analysis with at least one acceptable ladder may be selected.

### **5.4 Reviewing GeneMapper® ID-X Analysis Summary**

Any sample or control listed as meeting all thresholds (designated as a green square) does not require a manual review.

Any control that is identified as not meeting one or more thresholds must be manually reviewed. Unacceptable controls must be left in the project for review during Primary Analysis.

### **5.5 Verifying the GS600 Internal Size Standard for Controls and FDDU Samples in GeneMapper® ID-X**

Refer to the Interpretation Guidelines section for the review of GS600 size standards.

If any control contains a yellow triangle or a red stop sign in the *SQ* column, the size standard

must be verified. If any sample contains a yellow triangle or a red stop sign in the *SQ* column, the size standard need only be verified if the flagged sample is being manually reviewed.

**5.5.1** Select all control(s) that have either a yellow triangle or a red stop sign present in the *SQ* column.

**5.5.2** If the flagged sample(s) are being manually reviewed, select all sample(s) that have either a yellow triangle or a red stop sign present in the *SQ* column.

**5.5.3** Select the “Size Match Editor” icon (icon with three red peaks) from the tool bar.

**5.5.4** Review all of the control/sample files verifying the GS600 peaks (See Figure 1 or 2 in Interpretation Guidelines).

**5.5.5** Override the *SQ* of any acceptable control/sample.

## **5.6 Examining Data in GeneMapper® ID-X**

**5.6.1** Highlight the control(s)/sample(s) requiring manual review. Select the “Display Plots” icon (icon with multi-colored peaks) from the tool bar. This will open the *Samples Plot* window.

**5.6.2** Peak labeling criteria are as follows:

- a. The 3730 and 3500xL Analysis Methods include a global filter that removes labels from peaks that are less than 16% of the peak height of the largest allele present at each locus.
- b. Peaks that do not fall within an allelic category are labeled “OL” allele (i.e., off ladder allele).
- c. Peaks that are less than the Peak Amplitude Threshold (PAT) specified within the GMIDX Analysis Method Editor are not labeled.

**5.6.3** Evaluate any control flagged by GMIDX. Refer to the Interpretation Guidelines section for Evaluation of Internal Size Standards, Allelic Ladders and Control Samples.

**5.6.4** If a control requiring manual review is deemed acceptable, the composite genotype quality of the control must be overridden.

**5.6.5** At the discretion of the Post PCR Reviewer, a general review may be performed by manually reviewing samples flagged by GMIDX to assess the overall quality of the data and whether the plate is acceptable and should be made available for Primary Analysis. If a general review is performed, proceed to step 5.6.11.

**5.6.6** At the discretion of the Post PCR Reviewer, a detailed review may be performed in addition to the general review. The detailed review includes manually reviewing samples flagged by GMIDX to determine if individual samples require rework. Each locus will be designated with a colored heading (green, yellow, or red). Loci designated as either yellow or red must be manually reviewed. Loci designated as green do not need to be reviewed.

**5.6.7** Unacceptable samples: Record the *Rework Reasons* and *Comments* (if applicable) for the sample(s) that are not acceptable for import into STACS and CODIS.

**5.6.8** All unacceptable samples are considered uninterpretable and must either be designated for rework using the STACS automatic rework function or removed/deleted from the GMIDX Project.

- a. To delete a sample from the project, during the manual review, check the “Mark Sample for Deletion” box on the right side of the plot window. When the sample plot window is closed, GMIDX will ask for confirmation that the samples should be deleted.
- b. To designate a sample using the STACS automatic rework feature, the following information must be entered into either the designated *User Defined* columns (e.g., UD1) of the *Samples* tab of the GMIDX Table or the STACS Rework Details form: *Point/Action*, *Rework Reason*, *Comments* (optional) and *Next Run* (optional).

**5.6.9** Select the “Genotypes” tab and verify that the following columns exist: *Sample Name*, *Marker*, *Allele 1*, *Allele 2*, *Allele 3*. If the STACS automatic rework functionality is being utilized, up to four *User Defined* columns (e.g., UD1) may also be included in the CODIS Table.

**5.6.10** As applicable, select “Export Table” from the *File* menu in the GMIDX tool bar.

**5.6.11** Save the GMIDX Project.

## **5.7 Exporting a GeneMapper® ID-X Project**

**5.7.1** In the “GeneMapper® ID-X Manager” select the project to be exported and click the “Export” button on the bottom of the window.

**5.7.2** Browse to the location where the project will be exported to and click “Save”.

## **5.8 Completing Post PCR Review**

**5.8.1** If a general review was performed, a CODIS table is not generated. Indicate the result of the review in STACS as successful, failed or aborted. Comments must be entered for plates with process failed results.

**5.8.2** If a detailed review was performed and a CODIS table was generated, import the file into STACS.

**5.8.2.1** *Control Verification:* STACS compares the expected and current profiles for the Blood/Buccal Internal Standards (BIS) and Combo Control (Negative).

**5.8.2.2** If an FDDU sample was deemed unacceptable and removed from the CODIS Table or designated for rework using the STACS automatic rework function, the *Sample ID* is displayed in the *Sample Rework* window.

**5.8.2.3** As applicable, select a *Rework Point*, *Protocol*, *Rework Reason*, *Rework Comment*, and *Next Run* entry for all the samples in the *Sample Rework* window and complete the activity.

## **6 Primary Analysis (Review/Interpretation)**

The genetic analysis data for all FDDU STR (Short Tandem Repeat) profiles must undergo primary analysis (interpretation) and secondary analysis (technical review) by a qualified FDDU Examiner.

Primary analysis may be performed by a FDDU Examiner at an offsite location that does not have access to the STACS software. In these instances, the FDDU Examiner must continue to follow all the steps in the procedures required for the review of FDDU data. However, the recording of the completion of primary analysis within STACS may be performed by the FDDU Examiner at a later date.

**6.1** Within STACS, select the plate to be analyzed.

### **6.2 Analyzing a GeneMapper® ID-X Project**

Refer to Section 5.2 for analyzing the sample files.

### **6.3 Allelic Ladder Analysis Summary**

This summary only appears if one or more of the allelic ladders have been identified as unacceptable by GMIDX.

Refer to the Interpretation Guidelines sections for the review of allelic ladders.

GMIDX analysis requires the presence of at least one allelic ladder in each project. Allelic ladders with yellow and/or red quality indicators must be evaluated.

Refer to Section 5.3 for evaluating allelic ladders.

## 6.4 Reviewing GeneMapper® ID-X Analysis Summary

Any sample or control listed as meeting all thresholds (designated as a green square) does not require a manual review.

Any control that is identified as not meeting one or more thresholds must be manually reviewed. Unacceptable controls must be left in the project for review during Secondary Analysis.

## 6.5 Verifying the GS600 Internal Size Standard for Controls and FDDU Samples in GeneMapper® ID-X

Refer to the Interpretation Guidelines section for the review of GS600 size standards.

If any control contains a yellow triangle or a red stop sign in the *SQ* column, the size standard must be verified. If any sample contains a yellow triangle or a red stop sign in the *SQ* column, the size standard must only be verified if the flagged sample is being manually reviewed.

Refer to Section 5.5 for verifying the GS600 peaks for controls and/or samples.

## 6.6 Evaluation of Potential Crosstalk

Refer to the Interpretation Guidelines section for the review of potential crosstalk (CT).

**6.6.1** Samples that appear to have CT peaks shall be reviewed to identify the source of the peak(s).

**6.6.2** Select the “Samples” tab. For each sample and/or control identified, highlight the identified sample as well as the sample(s) in the adjacent capillaries based on capillary number.

**6.6.3** Select the “Display Plots” icon (icon with multi-colored peaks) from the tool bar. Review the highlighted samples to ensure that crosstalk peaks have not been designated as true alleles.

## 6.7 Examining Data in GeneMapper® ID-X

**6.7.1** Highlight the control(s)/sample(s) requiring manual review. Select the “Display Plots” icon (icon with multi-colored peaks) from the tool bar. This will open the *Samples Plot* window. .

**6.7.2** Peak labeling criteria are as follows:

- a. The 3730 and 3500xL Analysis Methods include a global filter that removes labels from peaks that are less than 16% of the peak height of the largest allele present at each locus.

- b. Peaks that do not fall within an allelic category are labeled “OL” allele (i.e., off ladder allele).
- c. Peaks that are less than the Peak Amplitude Threshold (PAT) specified within the GMIDX Analysis Method Editor are not labeled.

**6.7.3** Each locus will be designated with a colored heading (green, yellow, or red). Loci designated as either yellow or red must be manually reviewed. Loci designated as green do not need to be reviewed.

**6.7.4** Evaluate any control flagged by GMIDX. Refer to the Interpretation Guidelines section for the Evaluation of Internal Size Standards, Allelic Ladders and Control Samples.

**6.7.4.1** If a control requiring manual review is deemed acceptable, the composite genotype quality of the control must be overridden.

**6.7.5** If necessary, evaluate the STR results for any FDDU sample(s) flagged by GMIDX. Refer to the Interpretation Guidelines sections for Evaluation of Peaks of Non-Genetic (Non-Allelic) Origin and Evaluation of FDDU DNA Samples.

**6.7.5.1** At the discretion of a FDDU Examiner, samples flagged must either be manually reviewed (to determine if the samples are acceptable), designated for rework using the STACS automatic rework feature or removed/deleted from the GMIDX project.

**6.7.5.2** The FDDU Examiner must review each flagged FDDU sample to examine the peak labels and edit the peak labels where/when necessary (e.g., labeling OL alleles, removing peak labels from a spike).

<u>Acceptable samples</u>	<p>Approved for import into STACS and CODIS. All labels must be removed (“Delete Alleles” option in GMIDX software) from peaks that are not attributable to DNA (e.g., spikes, bleed-through). Peak labels must also be removed from inconclusive loci. (i.e., one or more alleles &lt; PIT (peak interpretation threshold)).</p> <p><b>NOTE:</b> Hit confirmation samples and QA/QC samples with results <math>\geq</math> PAT (peak amplitude threshold) may be included in the CODIS table for import into STACS.</p>
<u>Off Ladder (OL) Alleles</u>	<p>If an off ladder allele is present in a sample, the peak label for the allele must be manually edited in GMIDX to include the letters “OL” before the number designation for the allele (e.g., OL&gt;10).</p> <p><b>NOTE:</b> Hit confirmation samples and QA/QC samples with OL alleles do not need to be labelled with a “OL”.</p>



<u>Peak Height Ratio (PHR) and Allele Imbalance (AI)</u>	<p>If the peak height ratio for alleles at a heterozygote loci is &lt;50% for Identifiler Direct and GlobalFiler Express, the peak label for the allele with lower RFU (relative fluorescence unit) value may be manually edited in GMIDX to include the letters “PH” (peak height) or “AI” (allele imbalance) before the number designation for the allele (e.g., AI23).</p> <p><b>NOTE:</b> Hit confirmation samples and QA/QC samples with PHR or AI do not need to be labelled with a “PH” or “AI”.</p>
<u>Tri-allelic (TRI) Patterns</u>	<p>If a sample has a tri-allelic pattern at a locus, the allele values that meet the peak interpretation threshold should be included in the CODIS Table and imported into STACS.</p>
<u>Unacceptable samples</u>	<p>Record the <i>Rework Reasons</i>, and <i>Comments</i> (if applicable) for the sample(s) that are not acceptable for import into STACS and CODIS. For samples flagged by GMIDX, but not manually reviewed, it is not necessary to make note of each Sample ID and a general <i>Rework Reason</i> (e.g., GMIDX Rework) may be recorded for all the samples.</p>

**6.7.5.3** If a sample requiring manual review is deemed acceptable, the composite genotype quality of the sample must be overridden.

**6.7.5.4** All unacceptable samples are considered uninterpretable and must either be designated for rework using the STACS automatic rework function or removed/deleted from the GMIDX Project. Samples flagged by GMIDX, but not manually reviewed, are unacceptable and must be removed/deleted from the GMIDX Project or designated for rework.

- a. To delete a sample from the project, during the manual review, check the “Mark Sample for Deletion” box on the right side of the plot window. When the sample plot window is closed, GMIDX will ask for confirmation that the samples should be deleted.
- b. To designate a sample using the STACS automatic rework feature, the following information must be entered into either the designated user defined columns (e.g., UD1) of the *Samples* tab of the GMIDX table or the STACS Rework Details form: *Point/Action*, *Rework Reason*, *Comments* (optional) and *Next Run* (optional).

**6.7.6** At the discretion of the FDDU Examiner, a CODIS Table may be generated at the completion of Primary Analysis for import into STACS resulting in sample(s) being sent back for rework. If a CODIS Table is not generated, proceed to step 6.7.9.

**6.7.7** Select the “Genotypes” tab and verify that the following columns exist: *Sample Name*, *Marker*, *Allele 1*, *Allele 2*, *Allele 3*. If the STACS automatic rework function is being utilized, up to four *User Defined* columns (e.g., UD1) may also be included in the CODIS Table.

**6.7.8** As applicable, select “Export Table” from the *File* menu in the GMIDX tool bar.

**6.7.9** Save the GMIDX Project.

## **6.8 Exporting a GeneMapper® ID-X Project**

**6.8.1** In the “GeneMapper® ID-X Manager” select the project to be exported and click the “Export” button on the bottom of the window.

**6.8.2** Browse to the location where the project will be exported to and click “Save”.

## **6.9 Completing Primary Analysis**

**6.9.1** If a CODIS Table was not generated, indicate the result of the Primary Analysis in STACS as successful, failed or aborted. Comments must be entered for plates with processed failed results.

**6.9.2** If a CODIS Table was generated, import the file into STACS.

**6.9.2.1** *Control Verification:* STACS compares the expected and current profiles for the Blood/Buccal Internal Standards (BIS) and Combo Control (Negative).

**6.9.2.2** If an FDDU sample was deemed unacceptable and removed from the CODIS Table or designated for rework using the STACS automatic rework function, the *Sample ID* is displayed in the *Sample Rework* window.

**6.9.2.3** As applicable, select a *Rework Point*, *Protocol*, *Rework Reason*, *Rework Comment*, and *Next Run* entry for all the samples in the *Sample Rework* window and complete the activity.

## **7 Secondary Analysis (Technical Review)**

The FDDU Examiner that performs the secondary analysis (technical review) must be different from the FDDU Examiner that performed the primary analysis (interpretation).

Secondary analysis may be performed by a FDDU Examiner at an offsite location that does not have access to the STACS software. In these instances, the FDDU Examiner must continue to follow all the steps in the procedures required for the review of FDDU data. However, the recording of the completion of secondary analysis within STACS and the import of the resulting CODIS Table may be performed by the FDDU Examiner at a later date.

**7.1** Within STACS, select the plate to be analyzed.

## 7.2 Importing and Reviewing a GeneMapper® ID-X Project

**7.2.1** Open the GMIDX software, if not already open and select “GeneMapper® ID-X Manager” from the *Tools* menu on the tool bar.

**7.2.2** From the *Projects* tab, click the “Import” button on the bottom of the window.

**7.2.3** Browse to the project that is to be imported. Highlight the project and select “Import”. Select “GeneMapper® ID-X Security Group” and select “OK”.

**NOTE:** If the project already exists in the GMIDX database, it does not need to be reimported.

**7.3** Click on the “Analysis Summary” tab and verify that all ladders, controls and/or samples flagged as “One or more thresholds not met” (designated by a red stop sign CGQ [Composite Genotype Quality]) have either been:

- (a) Reviewed and accepted following an edit and CGQ override in GMIDX (designated by a green stop sign CGQ); or
- (b) Designated for rework using the STACS automatic rework function; or
- (c) Deleted from the GMIDX project.

**NOTE:** Unacceptable controls (designated by a red stop sign) should have been left in the project during Primary Analysis so they are available for review by the FDDU Examiner performing Secondary Analysis.

**7.4** Click on the “Samples” tab, then “Project” in the navigation panel on the left side of the screen. This will bring up an unfiltered list of all samples in the project.

**7.5** Evaluate the GS600 Internal Size Standards for any ladder, control, and/or sample where the sizing quality was overridden during post PCR review or primary analysis as designated by a green check in the *SQO* (Sizing Quality Overridden) column. Refer to the Interpretation Guidelines section for the review of GS600 size standards.

**7.6** Evaluate the electronic data in GMIDX using the *Raw Data* tab for each Combo Control (Negative) to verify the presence of a primer peak in the raw data.

**7.7** Select the table setting “View CGQ Overrides”. This will display a filtered list of all ladders/controls/samples where the CGQ was overridden. This view only displays samples where the CGQ was overridden, (e.g., a sample containing a tri-allele at a locus was deemed acceptable and the marker overridden during Primary Analysis).

**7.7.1** Evaluate the STR results for all ladders/controls/samples that are listed. Refer to the Interpretation Guidelines sections for Evaluation of Peaks of Non-Genetic (Non-Allelic) Origin and Evaluation of FDDU DNA Samples.

**7.7.2** The FDDU Examiner must review each FDDU sample and/or control listed in the table “View CGQ Overrides” to examine the peak labels and review the previously recorded results to ensure that he/she is in agreement with the interpretations made during Primary Analysis.

**7.8** Select the table setting “View Edited Samples”. This will display a filtered list of all ladders/controls/samples where an edit was made to a specific allele call, (i.e., removal of a pull-up peak or renaming of an off-ladder allele during Primary Analysis).

**7.8.1** Evaluate the STR results for all ladders/controls/samples that are listed. Refer to the Interpretation Guidelines sections of Evaluation of Peaks of Non-Genetic (Non-Allelic) Origin and Evaluation of FDDU DNA Samples.

**7.8.2** The FDDU Examiner must review each FDDU ladder/control/sample listed in the table “View Edited Samples” to examine the peak labels and review the previously recorded results to ensure that he/she is in agreement with the interpretations made during Primary Analysis.

**7.9** Evaluate any buccal samples and/or controls that were previously recorded during Primary Analysis as having crosstalk peaks present. Refer to the Interpretation Guidelines section for the review of potential crosstalk.

**7.10 Unacceptable Samples:** Review any previously recorded *Rework Reasons* and/or *Comments* (if applicable) made during Post PCR Review of Primary Analysis (if applicable). For samples flagged by GMIDX, but not manually reviewed, it is not necessary to record each Sample ID and a general *Rework Reason* (e.g., GMIDX Rework) may be recorded for all the samples.

**7.11** At the discretion of the FDDU Examiner performing Secondary Analysis, additional FDDU samples deemed unacceptable may be designated for rework using the STACS automatic rework feature or removed/deleted from the project. Samples flagged by GMIDX, but not manually reviewed, are unacceptable and must be removed/deleted from the GMIDX Project.

**7.12** If there are interpretation discrepancies between the primary and secondary analysis, they must be resolved before the *Secondary Analysis* can be completed and the FDDU STR profiles are approved for import into STACS and CODIS.

### **7.13 Completing Secondary Analysis**

**7.13.1** Following completion of the secondary analysis (technical review), the FDDU Examiner must generate a CODIS table.

**7.13.1.1** Select the “Genotypes” tab and verify that the following columns exist: *Sample Name*, *Marker*, *Allele 1*, *Allele 2*, and *Allele 3*. If the STACS automatic rework function is being utilized, up to four *User Defined* columns (e.g., UD1) may also be included in the CODIS table.

**7.13.1.2** Select “Export Table” from the *File* menu in the GMIDX tool bar.

**7.13.1.3** If any modifications were made to the GMIDX Project, save the project and use the *GeneMapper® ID-X Manager* Export option to export the project into the existing local project folder that was copied from the STACS network at the beginning of Secondary Analysis.

**7.13.2** Import the CODIS Table into STACS.

**7.13.2.1 Control Verification:** STACS compares the expected and current profiles for the Blood/Buccal Internal Standards (BIS) and Combo Control (Negative). The results are displayed in the *Control Verifications Results* window. Control profiles that fail to exhibit the expected DNA typing results appear in red; failures must be recorded.

**7.13.2.2** If an FDDU sample was deemed unacceptable and removed from the CODIS Table or designated for rework using the STACS automatic rework function, the *Sample ID* is displayed in the *Missing Rework* list.

**7.13.3** As applicable, select a rework *Action*, *Protocol*, *Reason*, *Comment*, and *Next Run* for all the samples in the *Missing Rework* window.

**7.13.4** As applicable, review the results displayed on each STACS form displayed (e.g., Validation Rework, Invalid Allele, Hit Confirmation, Mismatch) and take the appropriate action to complete the activity.

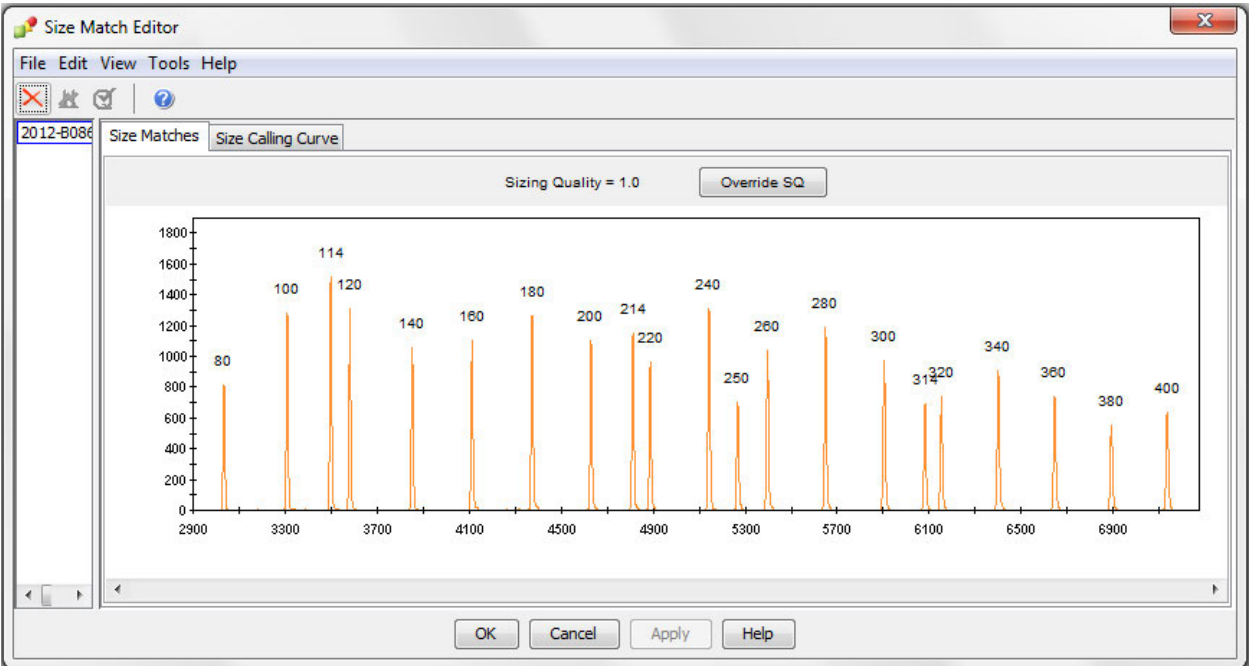
## 8 Interpretation Guidelines

The FDDU Examiner will use the following guidelines for interpretation of data. For any sample that the FDDU Examiner determines must be reworked, the data under review will be considered uninterpretable.

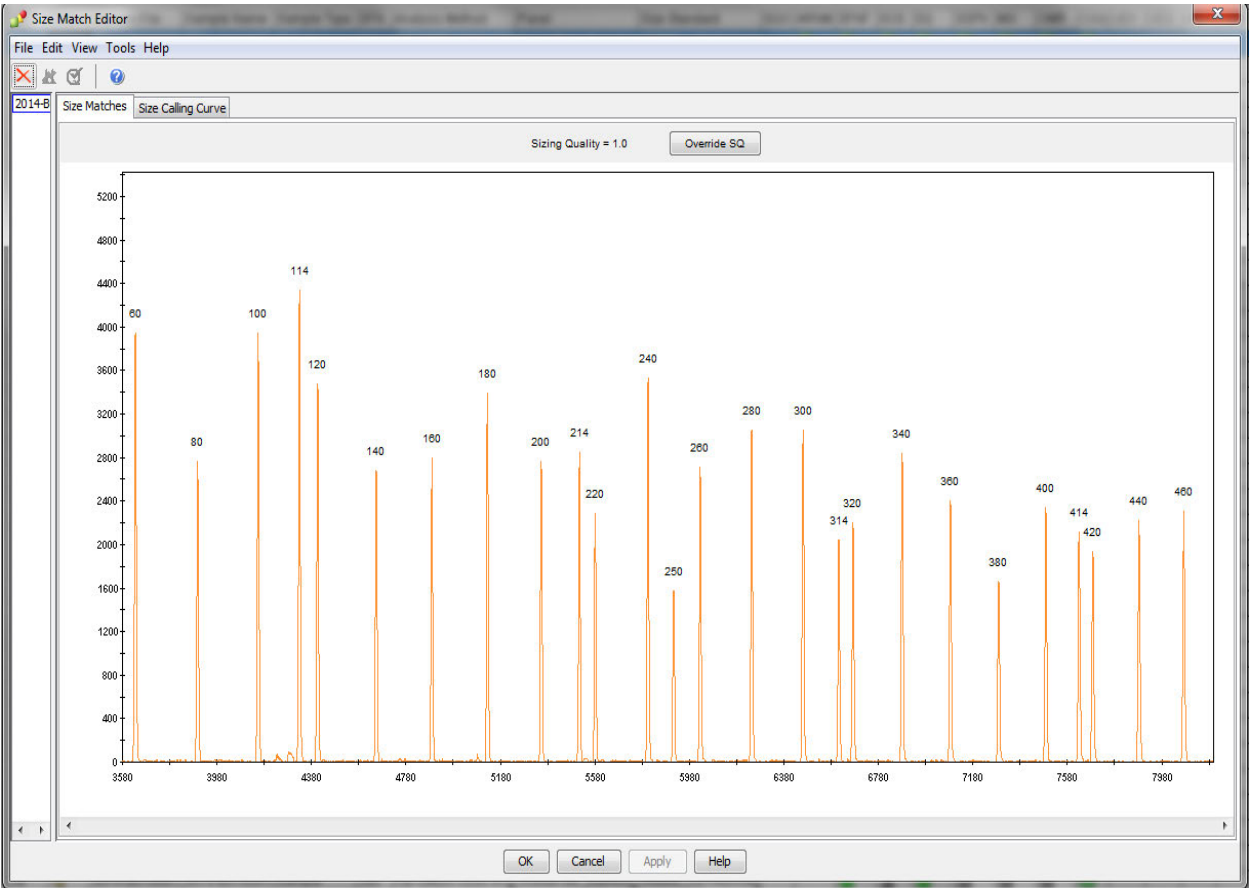
### 8.1 Evaluation of Internal Size Standards, Allelic Ladders and Control Samples

#### 8.1.1 Internal Size Standard: GS600 [LIZ]

Verify the correct fragments are captured and have been assigned the correct size values for all FDDU samples, controls, and allelic ladders. See Figures 1 and 2.



**Figure 1 – GS600 Size Standard (IDD)**



**Figure 2 – GS600 v2.0 Size Standard (GFE)**

### 8.1.2 Allelic Ladders

The Identifiler Direct and/or GlobalFiler Express Allelic Ladder, which is run separately from any sample(s) or control(s), is used by the GMIDX software as a reference for designating alleles. Each allelic ladder is a kit reagent that consists of amplified allelic fragments of known size and repeat content. These ladders do not contain all possible alleles that could be detected at an individual STR locus (i.e., off-ladder alleles). Any allelic ladder(s) used for comparison and assignment of allele designations must exhibit the correct allele designations in GMIDX. See Figure 3 and 4. The Y indel, pictured in the green channel of Figure 4, is not interpreted and therefore will not be labeled in the GMIDX software.

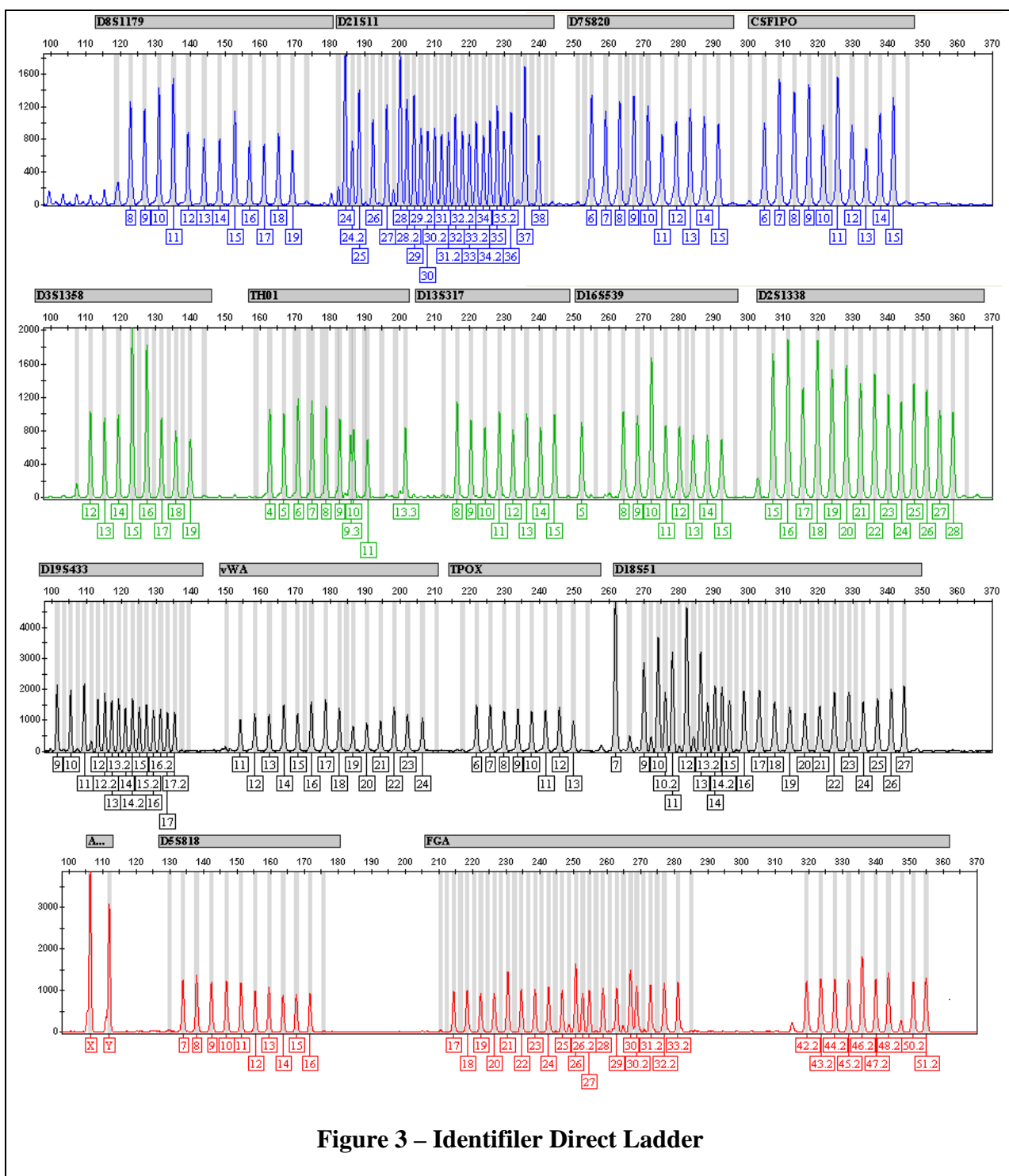
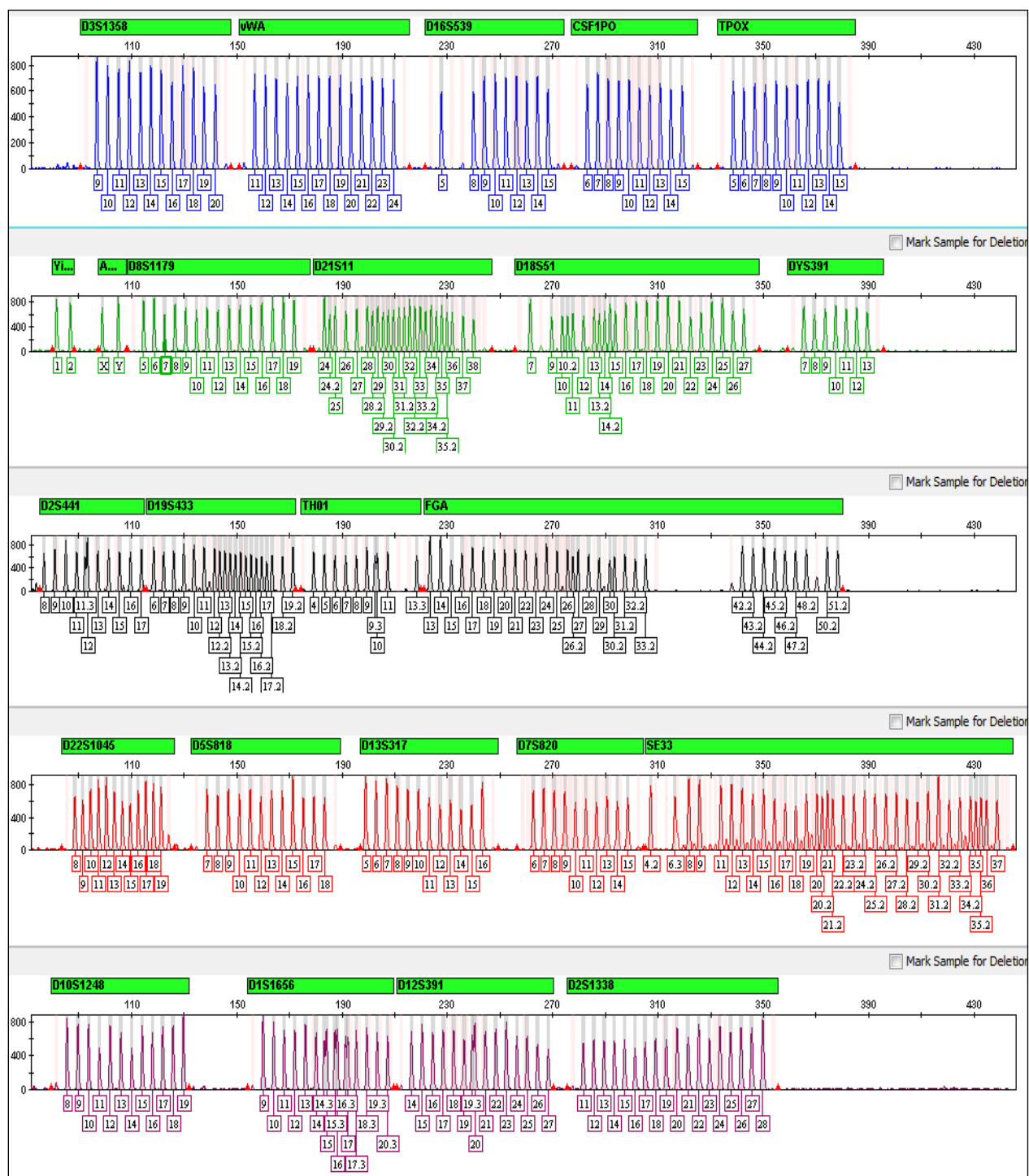


Figure 3 – Identifiler Direct Ladder





**Figure 4 – GlobalFiler Express Ladder**

### 8.1.3 Blood/Buccal Internal Standard (BIS)

A Blood/Buccal Internal Standard is a positive control for both the STR loci and amelogenin sex typing locus. Two (2) BIS controls must be included with each 96 Well plate of FDDU samples punched. The BIS control is processed through the same extraction and/or amplification and electrophoretic typing procedure as the FDDU samples and is used to evaluate the performance of the entire FDDU DNA analysis procedure. Any combination of BIS controls from approved donors may be used. The DNA typing results for the BIS controls are verified by STACS during Secondary Analysis.

**8.1.3.1** At minimum, one BIS control included on each plate must yield acceptable DNA typing results that exhibit all the expected allelic peaks ( $\geq$  PAT) and must not exhibit any extraneous allelic peaks.

**8.1.3.2** If one BIS control is acceptable and exhibits the expected DNA typing results, but the second BIS control yields unacceptable results (e.g., injection failure,  $<$  PAT, loss of resolution, off scale), no action is required. However, it should be verified that all loci with designated allele values are consistent with the expected STR typing results for the BIS control by leaving the BIS control in the GMIDX project and allowing the type to be verified by STACS during Secondary Analysis.

**8.1.3.3** If both BIS controls yield unacceptable DNA typing results, the following steps must be taken (as applicable):

- a. If there appears to be an injection or electrophoretic problem (e.g., injection failure, loss of resolution, off scale data, excessive bleed-through), re-inject the BIS control(s), or, if necessary, re-inject the entire plate.<sup>1</sup> If re-injection of the BIS control(s) does not resolve the problem, all FDDU samples associated with the BIS control(s) must be re-worked.
- b. If there does not appear to be an injection or electrophoretic problem, all FDDU samples associated with the BIS control(s) must be re-worked.<sup>2</sup>

**8.1.3.4** If one or both BIS controls are acceptable, but fail to exhibit the expected DNA typing result, the following steps must be taken (as applicable):

- a. If there appears to be an injection or electrophoretic problem (e.g., mobility shift, loss of resolution), re-inject the BIS control(s), or, if necessary, re-inject the entire

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<sup>1</sup> If the BIS control(s) exhibited off scale data, they may be re-injected with less DNA (i.e., by reducing the injection time).

<sup>2</sup>As used in this procedure, “re-worked” refers to sending the sample(s) back to the STaCS Plate Creation module so they can be assigned to a new plate and re-processed through the entire FDDU DNA Analysis procedure (e.g., STR amplification, Capillary Electrophoresis).

plate. If re-injection of the BIS control(s) does not resolve the problem, all FDDU samples associated with the BIS control(s) must be re-worked.

- b. If there does not appear to be an injection or electrophoretic problem, all FDDU samples associated with the BIS control(s) must be re-worked.

It should be verified that all loci with designated allele values are consistent with the expected STR typing results for the BIS control by leaving the BIS control in the GMIDX project and allowing the type to be verified by STACS during Secondary Analysis.

#### **8.1.4 Combo Control (NEGATIVE)**

Two (2) Combo controls, designated as a NEGATIVE in STACS, must be included with each 96-Well plate amplified with Identifiler Direct or GlobalFiler Express. The Combo control is a combination of the Negative and Amplification Blank controls for the amplification process. This control contains all the reagents used in the pre-amplification and amplification process minus a sample punch and is processed through the same plate preparation, amplification and electrophoretic typing procedures as the FDDU samples. The Combo control functions as a reagent blank control for detection of potential DNA contamination(s) that may have been introduced in the pre-amplification or amplification reagents. The expected outcome is that no DNA typing results are obtained for the Combo control(s). The detection of allelic peak(s) in the Combo control(s) is indicative of the potential presence of adventitious DNA in the reagents and/or supplies used during pre-amplification and/or DNA amplification. The source of the adventitious DNA can be non-amplified DNA or amplified PCR product.

**8.1.4.1** If one Combo control is acceptable and the second Combo control exhibits unacceptable results indicative of an injection or electrophoretic problem (e.g., injection failure, loss of resolution) no action is required.

**8.1.4.2** If a Combo control exhibits a peak(s)  $\geq$  PAT within the expected size range of the AmpF/STR loci that are not attributable to a spike or other artifact (e.g., raised baseline, dissociated primer dye, crosstalk), all FDDU samples extracted and/or amplified with the reagents contained in the Combo control must be re-worked.

**8.1.4.3** At the discretion of a FDDU Examiner, Combo controls containing spikes or other artifacts (e.g., raised baseline, dissociated primer dye) within the expected size range of the AmpF/STR loci may be re-injected. Spikes or other artifacts that fall outside the expected size range are not significant.

- a. Subsequent injections of the Combo control that also exhibit spikes need not be re-injected if the original and subsequent spikes have different base pair sizes.
- b. If there is more than one Combo control included with the FDDU samples and: 1) Both controls have spikes, but they have different base pair sizes -OR- 2) One

control has a spike that is not present in the other Combo control(s); the samples need not be re-injected.

**8.1.4.4** For each electrophoretic run of a Combo Control, the electronic data must be reviewed by a FDDU Examiner in GMIDX to verify the presence of a primer peak in the raw data.

## **8.2 Evaluation of Peaks of Non-Genetic (Non-Allelic)<sup>3</sup> Origin**

Non-genetic data may be undesired PCR products (e.g., stutter and non-template dependent nucleotide addition), analytical artifacts (e.g., spikes, raised baseline), instrumental limitations (e.g., pull-up, bleed-through, and crosstalk), or be introduced into the process (e.g., disassociated primer dye). The various types, and evaluation, of these non-genetic peaks are described below.

### **8.2.1 Stutter**

Stutter is a minor peak product that is inherent to the PCR amplification of repetitive DNA sequences (e.g., STR loci). Stutter peaks originate as the template DNA is being copied during the PCR reaction and do not constitute an individual's genotype. Stutter is most often observed as a minor PCR product one repeat unit smaller (e.g., -4 bp in a tetranucleotide STR locus) than the source allelic peak. Stutter is expressed as a percentage relative to the source allelic peak (i.e., % Stutter). The GMIDX analysis methods have a 20% filter that removes any stutter peaks less than 20% of the corresponding allele. At the discretion of a FDDU Examiner, FDDU samples exhibiting stutter >20% at a locus may be re-worked.

### **8.2.2 Non-Template Dependent Nucleotide Addition**

Taq DNA Polymerase is known to add an additional nucleotide (i.e., typically "A") to the 3' ends of double-stranded PCR products in a non-template dependent manner. This phenomenon is called "adenylation" and results in the generation of the "plus-A" fragment. This plus-A fragment is the allele (N) produced by the PCR amplification. A minority of amplified fragments do not have this additional nucleotide added, these "minus-A" fragments are one bp shorter (N-1) than the allelic fragment. The generation of minus-A fragments is sequence dependent and thus kit and locus specific. Minus-A is expressed as a percentage relative to the allelic peak (i.e., % minus-A). At the discretion of a FDDU Examiner, FDDU samples exhibiting minus-A >16% on the 3730 or 3500xL at a locus may be re-worked.

### **8.2.3 Spikes**

**8.2.3.1** Spikes are non-specific, non-reproducible peaks that may result during capillary electrophoresis. Spikes may be caused by electrical fluctuations in the power source, urea crystals, or bubbles in the polymer. Spikes are generally detected in two or more colors and size

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<sup>3</sup>Non-genetic or Non-specific peaks are those that do not represent (i.e., do not have their origin in) allelic STR information.

within  $\pm 0.13$  bp ( $\pm$  approximately one scan unit) of each other; however, spikes that occur in only one color have been observed.

**8.2.3.2** At the discretion of a FDDU Examiner, FDDU samples exhibiting spikes within the expected size range of the AmpF/STR<sup>®</sup> loci may be re-injected or re-worked. However, if a spike occurs at or sufficiently close to an internal size standard peak or an allelic peak such that sizing and/or allelic designation is affected, the FDDU sample(s) must be re-injected or re-worked. Subsequent runs of the sample that also exhibit spikes need not be re-injected or re-worked if the original and subsequent spikes have different base pair sizes.

#### **8.2.4 Raised Baseline**

Raised baseline appears in an electropherogram as a non-specific elevation of the baseline (horizontal axis) between one or more peaks. This elevation may result from excessive template DNA or be instrument related. At the discretion of a FDDU Examiner, samples exhibiting a raised baseline may be re-injected or re-worked.

#### **8.2.5 Pull-Up**

The excessive fluorescence intensity associated with an off scale peak may result in the appearance of a lower intensity peak that is generally observed in the color channels spectrally adjacent to that of the off scale peak. The resulting artifact is called a pull-up peak and typically sizes within  $\pm 0.25$  bp ( $\pm$  approximately two scan units) of its source peak. At the discretion of a FDDU Examiner, samples exhibiting pull-up may be re-injected with less DNA (i.e., by reducing the injection time) or re-worked.

#### **8.2.6 Bleed-Through**

A bleed-through peak is similar to a pull-up peak (i.e., it appears in a spectrally adjacent color and sizes within  $\pm 0.25$  bp of its source peak). However, the source of the bleed-through peak is not off scale data. At the discretion of a FDDU Examiner, samples exhibiting bleed-through may be re-injected or re-worked.

#### **8.2.7 Dissociated Primer Dye**

Dye peaks may result from dye that has become dissociated from the primers present in some lots of the AmpF/STR<sup>®</sup> kits and migrate independently during capillary electrophoresis. Since the dissociated dye originates from a kit reagent, dye peaks that exceed the GMIDX PAT may be detected in any sample or control. Dye peaks possess the spectrum of one of the dyes in the AmpF/STR<sup>®</sup> kit(s) and a broader morphology than that of allelic peaks. At the discretion of a FDDU Examiner, samples exhibiting dye peaks may be re-injected or re-worked.

## 8.2.8 Crosstalk

Crosstalk between adjacent capillaries can be observed when one capillary contains a low quantity of DNA and an adjacent capillary contains a high quantity of DNA that results in a large amount of fluorescence being captured by the CCD (Charge Coupled Device) camera. The high level of fluorescence and the proximity of the capillaries can cause a low level of fluorescence to appear in an adjacent capillary. The level of CT can be higher for buccal samples than for blood samples. At the discretion of an FDDU Examiner, samples exhibiting crosstalk may be re-injected or re-worked.

## 8.3 Evaluation of FDDU DNA Samples

### 8.3.1 Peak Interpretation Threshold (PIT) and Peak Amplitude Threshold (PAT)

**8.3.1.1** The PIT for FDDU samples is  $\geq 150$  RFU for IDD or  $\geq 175$  RFU for GFE.<sup>4</sup> STR loci with peaks  $<$  PIT are considered inconclusive and ineligible for entry into STACS and CODIS.

**8.3.1.2** The PAT for FDDU samples is  $\geq 50$  RFU for IDD or  $\geq 75$  RFU for GFE. Peaks  $<$  PAT are not labeled during GMIDX analysis.

**8.3.1.3** If labeled peaks  $\geq$  PAT indicate the potential presence of a mixture or contamination (adventitious DNA), the affected sample(s) and/or control(s) must be re-worked.

### 8.3.2 Excessive DNA Template and Off Scale Samples

**8.3.2.1** An excessive amount of template DNA may result in the appearance of off scale peaks. These samples may also exhibit raised baseline, pull-up peaks, elevated stutter and/or incomplete non-template-dependent nucleotide addition (-A) peaks, and/or non-specific peaks in one or more colors. Samples with an excessive amount of DNA template may also exhibit the similar characteristics, but in the absence of off scale peaks.

**8.3.2.2** At the discretion of a FDDU Examiner, samples that exhibit the characteristics described above (both in the presence and absence of off scale peaks), may be re-injected with less DNA (i.e., by reducing the injection time) or re-worked.

### 8.3.3 Interpretation of Single Source FDDU DNA Samples

**8.3.3.1** A sample is considered to have originated from a single individual if only one or two alleles are present at all loci for which typing results were obtained,<sup>5</sup> and the peak height ratios

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<sup>4</sup>The 3730 and 3500xL Analysis Methods include a global filter that removes labels from peaks that are less than 16% of the peak height of the largest allele present at each locus.

<sup>5</sup>Individuals can display three allelic peaks at a locus (see *Tri-allelic Patterns* section). Samples in which three allelic peaks at a locus have been observed and confirmed may be concluded to be single-source if no other typing

for all heterozygous loci are within the expected value<sup>6</sup> (see *Peak Height Ratios and Allele Imbalance* section).

**8.3.3.2** If a sample exhibits more than two alleles at two or more loci and/or the peak height ratios between the allelic peaks for more than one locus are below the expected value, the sample shall be handled as a possible mixture and re-worked.

### **8.3.4 Peak Height Ratios and Allele Imbalance**

For alleles at a heterozygote locus, Peak Height Ratios (PHR) are determined by dividing the peak height of the allele with the lower RFU value by the peak height of the allele with the higher RFU value, and then multiplying this value by 100 to express the PHR as a percentage. The expected PHR for a heterozygote locus is  $\geq 50\%$  for IDD and GFE.

**8.3.4.1** Allele (peak height) imbalance may be seen in the typing results from a single individual due to elevated stutter, primer binding site variants that result in attenuated amplification of one allele of a heterozygous pair, tri-allelic patterns, etc. Samples exhibiting a PHR  $< 50\%$  for IDD and GFE at more than one of the CODIS Core Loci must be re-worked for confirmation. At the discretion of a FDDU Examiner, samples exhibiting a PHR  $< 50\%$  for IDD and GFE at one of the CODIS Core Loci may be re-worked for confirmation. At the discretion of a FDDU Examiner, a non-CODIS Core locus exhibiting a PHR  $< 50\%$  (as applicable) may be considered inconclusive and ineligible for entry into STACS and CODIS.

### **8.3.5 Off Ladder (OL) Alleles**

**8.3.5.1** Using alleles that are present in the allelic ladder, GMIDX creates allele size categories that are centered on the size of the allelic ladder peaks. Additionally, GMIDX creates categories for “virtual” alleles (ones not physically present in the allelic ladder and thus have no corresponding peaks in the electropherogram of the allelic ladder) at some loci. Off-ladder (OL) alleles are those DNA fragments that fail to size within an allele category.

**8.3.5.2** OL alleles that fall between alleles physically present within the allelic ladder will be designated in accordance with guidelines of the International Society for Forensic Haemogenetics. To determine the allelic designation, OL peaks are converted to size in base pairs (bp) and compared to the size of the flanking ladder alleles. If the OL allele is not an integer tetranucleotide repeat (e.g., 12, 13, 14), but rather varies by 1, 2, or 3 bp from a ladder allele, then it will be designated as a microvariant of the smaller ladder allele that flanks the OL allele. For example, if an OL allele occurs between the allele size categories that represent alleles 12 and 13, and it is approximately 1 bp larger than the 12 ladder allele, the OL allele is designated a 12.1.

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results indicate the presence of a mixture.

<sup>6</sup>Allele (peak height) imbalances may be seen in the typing results from a single individual (see *Peak Height Ratios and Allele Imbalance* section). Samples in which allele imbalance at a locus has been observed and confirmed may be concluded to be single-source if no other typing results indicate the presence of a mixture.

Alternatively, the off ladder allele can be designated by performing a visual interpretation of the allele using the *Show Bin* function in GMIDX.

**8.3.5.3** If an OL allele does not fall within the size range of any locus-specific allelic ladder, it must be associated with one of the two allelic ladders between which it falls.

- a. If the OL allele is flanked by a locus with two peaks (i.e., heterozygous locus) and a locus with a single peak, the OL allele is assigned to the latter locus.
- b. If both loci between which an OL allele falls each display either a single allele or two alleles, the OL allele may be assigned to the locus closest in size to the OL allele.<sup>7</sup>

**8.3.5.4** If an OL allele falls above the largest or below the smallest physically present ladder allele, the OL allele will be designated as either greater than (>) or less than (<) the respective ladder allele (e.g., >30).

**8.3.5.5** Samples exhibiting OL alleles at the CODIS Core Loci must be re-worked for confirmation. At the discretion of a FDDU Examiner, a non-CODIS Core locus exhibiting an OL allele may be considered inconclusive and ineligible for entry into STACS and CODIS.

**8.3.5.6** The allelic ladder may be periodically updated with additional “virtual” allele size categories. If this takes place during a sample’s confirmation run, the GMIDX software may no longer identify the allele as off ladder. In these instances, it may be necessary for the FDDU Examiner to manually edit the allele in GMIDX to include the “OL” label. This designation is needed for STACS to complete the confirmation run and allow the sample to be uploaded to CODIS.

### **8.3.6 Tri-Allelic Patterns**

Individuals can display three allelic peaks at a locus. Observed tri-allelic patterns are recorded at [http://www.cstl.nist.gov/biotech/strbase/tri\\_tab.htm](http://www.cstl.nist.gov/biotech/strbase/tri_tab.htm).

**8.3.6.1** Samples exhibiting a tri-allelic pattern at the CODIS Core Loci should be re-worked for confirmation. If the tri-allelic pattern is confirmed, the allele values for the labeled peaks that meet the PIT will be imported into STACS and subsequently CODIS. At the discretion of a FDDU Examiner, a non-CODIS Core locus exhibiting a tri-allelic pattern may be considered inconclusive and ineligible for entry into STACS and CODIS.

**8.3.6.2** A peak in a tri-allelic pattern may have a lower RFU value than the sister peaks at that locus, which may either fall below the PAT or be filtered out by GMIDX during the confirmation process.

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<sup>7</sup> To facilitate the interpretation of OL alleles, the FDDU Examiner may consult a listing of such alleles recorded at [http://www.cstl.nist.gov/div831/strbase/var\\_tab.htm](http://www.cstl.nist.gov/div831/strbase/var_tab.htm).



**8.3.6.2.1** At the discretion of a FDDU Examiner, if the third allele appears again during the confirmation process, the allele designation may be removed to allow the remaining peaks at that locus to be entered into STACS and subsequently CODIS.

**8.3.6.2.2** If this third allele is  $\geq$ PIT, but filtered out by GMIDX during the confirmation run, the FDDU Examiner may manually add the allele label to the peak so that the verification can be completed in STACS.

### **8.3.7 Amelogenin**

A specimen of male origin will exhibit both the X and Y amelogenin fragments, whereas a specimen of female origin will exhibit only the X fragment. However, primer binding site variants may occur at the amelogenin genetic locus that result in peak height ratios of significantly  $<50\%$  between the X and Y fragments. In these instances, the X peak may be filtered out or completely absent from the electropherogram, giving the appearance of a Y,Y result. In these instances the Y,Y result will be imported into STACS and subsequently uploaded to CODIS (Amelogenin results are uploaded to CODIS, but are not searched).

## **9 Calculations**

### **9.1 Stutter**

Stutter is most often observed as a minor PCR product peak. Stutter is expressed as a percentage relative to the source allelic peak.

$$\% \text{ Stutter} = \text{Peak Height (stutter peak)} / \text{Peak Height (source allelic peak)} \times 100$$

### **9.2 Non-Template Dependent Nucleotide Addition (-A)**

Minus-A fragments are one base pair (bp) shorter (N-1) than the allelic fragment (N). Minus-A is expressed as a percentage relative to the allelic peak (i.e., % minus-A).

$$\% \text{ Minus A} = \text{Peak Height (N-1)} / \text{Peak Height (N)} \times 100$$

### **9.3 Peak Height Ratios (PHR)**

For alleles at a heterozygote locus, the PHR is expressed as a percentage:

$$\text{PHR (\%)} = \text{Peak Height (allele with lower RFU value)} / \text{Peak Height (allele with higher RFU value)} \times 100$$

## **10 Limitations**

The FDDU Examiner that performs the secondary analysis (technical review) must be different from the FDDU Examiner that performed the primary analysis (interpretation).

## **11 Sampling or Sample Selection**

Not applicable.

## **12 Measurement Uncertainty**

Not applicable.

## **13 Safety**

Not applicable.

## **14 References**

The procedures described here are derived from a variety of sources. Portions of the protocol come directly from some of the references cited below.

Quality Assurance Standards for DNA Databasing Laboratories, Current version.

DNA Procedures Manual.

FBI Laboratory Quality Assurance Manual.

National Research Council. The Evaluation of Forensic DNA Evidence. Washington, DC, National Academy Press (1996).

STACS DNA Inc. *Sample Tracking and Control System (STACS) User's Guide*.

Applied Biosystems. GeneMapper® ID-X Software Version 1.3 Getting Started Guide, Foster City, CA.

Applied Biosystems. AmpF/STR® Identifiler® Direct PCR Amplification Kit User Guide.

Applied Biosystems. *GlobalFiler® Express PCR Amplification Kit User's Manual*. Applied Biosystems, Foster City, CA.

Applied Biosystems. *GlobalFiler® Express PCR Amplification Kit User Guide*. Applied Biosystems, Foster City, CA.

Rev. #	Issue Date	History
7	02/28/18	1 Updated scope 5.3.2 Updated Identifier panel 6.7.5.2 Added notes about label editing exemptions 8.3.5.2 Added “physically present” within the “allelic” ladder 8.5.5.6 Added to address additional virtual bins 14 Updated reference to DNA Procedures Manual
8	10/28/20	Removed Identifier and 3130xl throughout. Deleted Identifier controls no longer applicable throughout. 2: Updated STACS and GMIDX versions 4.2: Added clarification on quarterly timing. 5.6.7 and 6.7.5.2: Removed to record plate ID and sample ID 5.6.8 and 6.7.5.4: Added are uninterpretable 5.6.8 b. and 6.7.5.4 b.: Clarified info entered into either GMIDX or STACS 7.10: Changed to review any previous rework reasons 8: Added clarification for uninterpretable data 8.1.2: Added that the Y indel is not labeled in the software Removed sections 8.1.4, 8.1.6, & 8.1.7 describing Identifier controls no longer in use. Renumbered Combo Control section. 8.3.4: Removed Identifier peak height ratio percentage 8.3.7: Changed to <50% 14: Added GFE user guides

### Approval

Redacted - Signatures on File

DNA Technical Leader

Date: 10/27/2020

FDDU Chief

Date: 10/27/2020